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TIBIAL BONE DENSITY, CROSS-SECTIONAL GEOMETRY AND STRENGTH IN FINNISH PET RABBITS: A PERIPHERAL QUANTITATIVE COMPUTED TOMOGRAPHY STUDY

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ABSTRACT

Rabbit bones are brittle and prone to fissure formation. Radiographs of very young and old rabbits are often indicative of decreased bone density. The aim of this study was to investigate the tibial bone parameters in pet rabbits, and their association with age, sex, castration and dental disease. Eighty-seven (43 female/ 5 spayed, 44 male/19 castrated) pet rabbits (mean age 2.6 years, range 0.3-9.3 years) of various breeds were studied, of which 37 had dental disease. Right tibiae were scanned with peripheral quantitative computed tomography at the distal (4 %) and mid-shaft sites (50 % of the tibial length). Analysed bone parameters included the total cross-sectional area, cortical bone area and density, trabecular bone density and strength-strain index. The mean diaphyseal cortical density was high (about 1400 mg/cm^{3}) in comparison to many other species. Within the studied age range, age was weakly but positively associated with diaphysial cortical density, with the juvenile rabbits clearly showing the lowest values. There was no tendency for age-related decrease in trabecular or cortical bone density at least up to 6 years of age. Neither were sex, castration nor dental disease associated with decreased tibial bone density.

Keywords: Bone density, Neutering, Dental disease, Osteoporosis, Pet rabbit

INTRODUCTION

The rabbit bones are brittle (Oni and others 1988) and their skeleton is light in terms of total body weight. It accounts for only 6-8% of the body weight, whereas in a similarly sized cat it is approximately 13% (Hunt 2013). The skeleton in humans is 15-18% of normal body weight (Mitchell and others 1945, Forbes and others 1953). Rabbits have well-developed musculature in the hind limbs designed for sprinting, and as prey animals, rabbits are evolved for quick escapes. Collectively, these features may increase the risk of fracture, especially in cases when bone mineral density is decreased. Bone density measurement is currently an uncommon practice in veterinary medicine, although radiographs of very young and old rabbits, and of those fed on a muesli mixture without exposure to sunlight, can be indicative of decreased bone density (Langley-Hobbs and Harcourt-Brown 2013). Evaluation of bone mineral density based on plain radiographs lacks sensitivity while the inter-observer variation between radiologists is high (Wagner and others 2005). Widely available dual-energy X-ray absorptiometry (DXA) is a routine non-invasive clinical method for measuring bone mineral density and diagnosing osteoporosis in humans (Cummings and Melton 2002). However, the DXA results are difficult to interpret in physical terms. They are confounded by bone and body size, and no three-dimensional information on bone structure is available (Sievänen 2000). Computed tomography (CT) is commonly used in clinical bone imaging because of its ability to reconstruct the bones in multiple planes for three-dimensional analysis. In bone research, peripheral quantitative computed tomography (pQCT), high-resolution pQCT

(HR-pQCT) and micro-CT are more widely used than conventional CT scanners as the radiation dose is smaller, the scanners are freely movable, cheaper, bone parameters are received from the automated software and the measurements can be obtained from the bones of small animals, such as rodents. In the present study, pQCT was used because it measures cross-sectional area and geometry separately in the cortical and trabecular compartments. This gives more meaningful results for research purposes than from DXA (Sievänen and others 1998). The pQCT scanner used in this study is primarily designed for the measurement of bone parameters at distal radius and tibia in humans. The tibia was chosen as the target bone because it can be easily accessed in mildly sedated pet rabbits. The anatomy of the tibia provides both trabecular bone and thick cortical bone for measurements. It is also a well-established site of measurement in experimental and clinical bone research (Jämsä and others 1998, Sievänen and others 1998, Binkley and Specker 2000, Rantalainen and others 2013), and reported as most common site for limb fracture in pet rabbits (Sasai and others 2015).

In humans, menopausal oestrogen deficiency and low androgen levels after bilateral orchiectomy are the main endocrinological factors known to predispose the skeleton for decreased bone density (Järvinen and others 2003a, Vanderschueren and others 1992). Rabbits are widely used as experimental models for osteoporosis studies. Experimentally osteoporosis is easily and rapidly induced through ovariectomy alone (or in combination with low-calcium diet) or corticosteroid medication alone (or in combination with ovariectomy) (Baofeng and others 2010, Kamran and others 2010, Sevil and Kara 2010, Castaneda and others 2008). In female pet rabbits, ovariohysterectomy is recommended due to the high prevalence of uterine adenocarcinomas in older females, whereas most male pet rabbits are castrated to manage hormone-related behaviours, such as fighting and urine marking (Greene 1941, Ingalls and others 1964, Harcourt-Brown 2013, Künzel and others 2015, Harcourt-Brown 2017). So far it is not known if neutering confers long-term detrimental consequences in bone mineral density in pet rabbits and predisposes them to fractures. It is also speculated whether endogenous glucocorticoids caused by stress could induce bone changes in pet rabbits (Jekl and Redrobe 2013).

Dental disease is one of the most common health problem and cause for veterinary treatment in pet rabbits (Harcourt-Brown 2007, Jekl and Redrobe 2013, Mäkitaipale and others 2015). Its aetiology is unclear but metabolic bone disease caused by vitamin D and calcium deficiency may be one possible predisposing factor. Vitamin D and calcium deficiency can cause secondary hyperparathyroidism and lead to reduced bone density, which should be measurable in various bones. Despite the well-documented translucency of the radiographs and prepared skulls of rabbits with dental disease, bone density studies are lacking (Harcourt-Brown 2007).

This study was undertaken to evaluate several pQCT-measured bone parameters of tibia in pet rabbits, including mineral density and to investigate the association of bone parameters with age, sex and castration. A preliminary investigation of the possible association between dental disease and bone parameters was also done by comparing the results of dentally healthy rabbits and those with dental disease. We hypothesised that the cortical bone mineral density in pet rabbits would be decreased, especially in very young and old animals, neutered animals and in those with advanced dental disease.

MATERIALS AND METHODS

Animals

This study was performed in 2012 and 2013 as part of the Pet Rabbit Health Research Project in Finland. The study was approved by the Animal Experiment Board of Finland (5562/04.10.03/2011). Pet rabbits, judged healthy by their owners and not in need for veterinary treatment, were recruited to the study by advertisements. Voluntary registration to the survey was done by email by the owners. The study was carried out at the Veterinary Teaching Hospital of the University of Helsinki.

Data on the rabbits' age, breed, sex, and previous history of lameness, fractures and pregnancy were obtained from internet-based questionnaires. After physical examination, the rabbits were weighed and sedated by subcutaneous injection of medetomidine 0.1 mg/kg (Cepetor; CP Pharma Handelsgesellschaft mbH) and ketamine 5 mg/kg (Ketaminol; Intervet International BV). Lateral skull radiographs were taken, the molar teeth and oral cavity were examined using an otoscope and the dentition was graded using the grading system of progressive syndrome of acquired dental disease (PSADD) proposed by Harcourt-Brown in 1997.The pQCT scanning was performed and blood samples were collected into plain tubes, EDTA tubes and heparin syringes from either cephalic or saphenous vein under sedation. After the procedures, atipamezole 0.25 mg/kg (Revertor; CP Pharma Handelsgesellschaft mbH) was given subcutaneously to reverse the effects of the medetomidine. Haematological and biochemical profiles as well as blood gas analysis were performed. Serum samples were frozen for later use. Results of the physical examination and radiographs were published previously by Mäkitaipale and others (2015).

Exclusion criteria were medications at the time of the study, pregnancy or nursing within 6 months before the study, previous fractures and lameness, perineal soiling or pododermatitis grade 3 or higher (Mancinelli and others 2014) at the time of the study. Rabbits with conditions affecting bone metabolism, such as chronic kidney disease diagnosed by elevated blood urea (> 12 nmol/l), creatinine (> 165 nmol/l) and nephroliths, were also excluded.

Peripheral quantitative computed tomography

The right tibia of all pet rabbits was scanned by pQCT (Stratec XCT 2000 Research+, Stratec Medizintechnik GmbH, Pforzheim, Germany). The length of the tibia from the tibiotarsal joint to the stifle joint was measured with a calliper. For the pQCT scanning, the sedated rabbits were placed in a dorsal recumbent position on a padded custom-made plastic platform, while the right hind limb (tibial plane) was horizontally in extension through the mid-gantry of the pQCT scanner (Fig 1). The limb was secured in this position, using the forearm holder provided by the manufacturer (Fig 2). Padding was used to adjust the holder for the rabbits.

The proper position for the scanning was defined by palpation and with the aid of the of scanner laser beam. The tibiotarsal joint was set as the reference (zero) position. Thereafter, two pQCT slices were scanned at the distal and shaft sites, corresponding to 4% and 50% of the length of the tibia (Fig 3). The voxel size was 0.2 x 0.2 mm^2, tube voltage 60 kV and the scanning speed was 20 mm/s. The cross-sectional pQCT images were analysed using automated Stratec software version 6.00B. Following the procedures for human scanning (Sievänen and others 1998), contour mode 2 and peel mode 2 (C2/P2) analysis was used for calculating the bone parameters of interest (see the specific list below) at the distal site and for the total bone cross-sectional area (TotA) at the shaft site. For the cortical density of the mid-shaft site, a density threshold of 710 mg/cm^3 was applied to separate cortical bone from adjacent tissues. A density threshold of 480 mg/cm^3 was applied for bone strength estimation at both sites. Specifically, the following bone parameters were determined: total bone cross-sectional area (TotA, mm^2), cortical bone cross-sectional area (CortA, mm^2), the ratio of CortA to TotA, trabecular bone density (TrabD, mg/cm^3), cortical bone density (CortD, mg/cm^3), and strength-strain index (SSI). The SSI denotes the density-weighted polar section modulus of the cortical geometry and describes the bone resistance to torsional loading. The pQCT was regularly auto-calibrated with the hydroxyapatite phantom provided by the manufacturer.

Statistical analysis

All statistical analyses were conducted with SPSS version 22 (IBM SPSS Corp., Armonk, NY, USA). The mean and standard deviation are given as descriptive values.

For the investigation of the possible association between dental disease and bone parameters, the rabbits were divided into two groups after physical and radiological examination. One group comprised dentally healthy rabbits with no signs of dental disease, and the second group comprised rabbits with dental disease. In addition, rabbits with dental disease were further divided into two groups. The first group, “initial stage”, consisted of rabbits with grade 2 dental disease and the other group, “advanced stage”, consisted of rabbits with grades 3-5 dental disease.

Analysis of variance (ANOVA) was first used to determine whether the clinical characteristics differed between the subgroups of interest. The intergroup differences in bone parameters were estimated with general linear models (GLMs), using body weight and age as covariates and the subgroups as factors. Factors included sex (M/F), neutering (yes/no), castration (yes/no) and dental disease (yes/no). Weight was taken into account because body mass is the most appropriate trait to control for variation in body size and subsequent variation in skeletal loading (Van Der Meulen and Carter 1995). In addition, age was also used as a covariate, given the wide variation in age within the subgroups. Univariate Pearson correlations between age, weight, tibial length and bone parameters were also analysed to determine the associations of the bone parameters with age and body size among healthy rabbits. P values <0.05 were considered statistically significant.

RESULTS

Eighty-seven pet rabbits were included in the study. The age range was from 0.3 to 9.3 (mean 2.6, SD 1.9) years. Nineteen rabbits were younger than one year, but only two were older than 8 years. Forty-three (49.4%) were females (5 spayed, 38 intact) and 44 (50.6%) were males (19 castrated, 25 intact). Two males were castrated 2 months before the study, three 4-7 months before, 11 at least 12 months before and information was missing in three cases. The rabbits represented 13 different breed groups, of which Dwarf Lop was the most common (n = 29) followed by mixed-breed rabbits (n = 19). Bone parameters from distal site were available from 85 and from shaft site from 76 rabbits.

Within all breed groups, the body weight varied from 1.0 to 6.2 (mean 2.3, SD 1.1) kg and tibial length from 68.1 to 127 (mean 89.2, SD 13.5) mm. The correlation between body weight and tibial length was very strong ($P<0.001$). At the distal site of the tibia, age was associated only with the strength-strain index. Both body weight and tibial length were significantly associated with all the other bone parameters, except cortical density (tibial length) and the CortA:TotA ratio (body weight) (Table 1). The observed associations were negative with the trabecular density and the CortA:TotA ratio. At the mid-shaft site, age was negatively associated with strength-strain index, but positively associated with cortical density. Both body weight and tibial length were positively associated with total cross-sectional area and the strength-strain index and negatively associated with the CortA:TotA ratio.

The weight, age and tibial length were similar between intact female and male rabbits. After controlling for body weight and age, there were no statistically significant group differences in bone parameters between intact females and males ($P>0.05$) (Table 2).

When the influence of castration on bone parameters was examined, there were no significant differences between castrated and intact males ($P>0.05$) (Table 3). The tibial shaft cortical density in relation to age among healthy intact and neutered pet rabbits is shown in Figure 4 and among all rabbits in Figure 5 (Fig 4, Fig 5). Cortical density was positively associated with age, as juvenile rabbits clearly showed the lowest values while there was no indication for age-related decrease in cortical or trabecular density.

Dental disease was diagnosed in 37 rabbits: 27 had grade 2 disease, three grade 3, six grade 4, and one grade 5. Of these, 22 were females (three spayed) and 15 males (four castrated). There were no significant intergroup differences in bone parameters between healthy rabbits and those with dental disease or of rabbits with initial and advance stage of PSADD ($P>0.05$) (Fig 5). Rabbits with dental disease were older (mean age 3.3 years) than healthy rabbits (mean age 2.1 years) ($P<0.05$).

DISCUSSION

We investigated bone mineral density and geometry at the distal tibia and tibial mid-diaphysis in pet rabbits. Specifically, the influence of age, sex, castration and dental disease on several bone parameters was assessed. We also consider that the present sample of pet rabbits represents of the rabbit population commonly seen in veterinary clinics as patients.

Contrary to our expectations, age was weakly but positively associated with cortical density at the tibial diaphysis among the healthy pet rabbits. Trabecular and cortical bone density showed no tendency to decline at least until the age of 6 years. The mean cortical density in the rabbit tibial shaft was very high, being approximately 1400 mg/cm^3 in our study. For comparison, in young female athletes and adult mice the mean pQCT-measured tibial cortical density was about 1100 mg/cm^3 and 1000 mg/cm^3, respectively (Jämsä and others 1998, Rantalainen and others 2013). Rabbits are known to achieve skeletal maturation between 24 and 32 weeks of age, defined as the closure of the epiphyseal growth plates (Norris and others 2001). Bone mineral density increases during skeletal maturation and continues 4–8 weeks after the closure of the growth plates (Norris and others 2001, Isaksson and others 2010). Bone density is, however, only one factor behind fracture risk. Total and cortical cross-sectional areas are important determinants of bone strength. In this study of pet rabbits, total area and strength-strain index seemed to decrease with ageing, which was likely due to the fact that the small-sized breeds with smaller-sized bones were overrepresented among the older rabbits. In humans, bone mass and density begin gradually to decrease with age, starting from the trabecular bone density at 20-30 years of age, while the total cross-sectional area increases (Lauretani and others 2008, Riggs and others 2008, Specker and others 2015). In women, cortical bone density and area decrease with age, and menopause leads to accelerated bone loss and increases the risk of fractures (Lauretani and others 2008, Riggs and others 2008, Uusi-Rasi and others 2008). In men, cortical density decreases slightly, but the larger total bone area and cortical area help to maintain bone resistance against bending forces (Riggs and others 2004, Lauretani and others 2008). These sex differences are believed to be caused by not only hormonal modulation, but also by differences in physical activity and nutrition. Currently, there are no publications about the influence of old age on bone mineral density in rabbits. The life expectancy of pet rabbits has increased in recent years; some may live over 10 years. Seven years, or even five years is usually considered as the geriatric age in small and medium-sized rabbits and 4-5 years in giant breeds, which have shorter life expectancies (Lennox 2010, Chitty 2014). Aging increases risk of health disorders and in rabbits, the risk is reported to be higher in rabbits over 3 years old, compared to younger ones (Mäkitaipale and others 2015). In the present study, however, there was no tendency for age-related decrease in trabecular or cortical bone density at least up to 6 years of age. Only five rabbits were older than 6 years which limited our opportunity to investigate the apparent impact of advanced age on bone parameters that manifest as reduced bone mineral density. The positive association between tibial cortical density and age may be explained by the age distribution of the rabbits in our study. The mean age was 2.6 years, and 14 rabbits were skeletally immature (under 40 weeks), whereas only 10 rabbits were over 5 years old and could be considered geriatric. None of the giant rabbits was over 4 years of age.

As for potential differences in bone parameters between intact females and males, no significant group differences were observed after controlling for body weight and age. Studies in rats and humans have shown higher cortical density in young females compared to males, which has been linked to oestrogen effects on bone (Järvinen and others 2003a, Riggs and others 2004). Oestrogen stimulates activation of vitamin D and enhances calcium uptake from the gut. During puberty, oestrogen deposits extra calcium in the bones as a reservoir for the developing foetal skeleton and subsequent lactation and results in increased cortical density. Stiffer bone, in turn, suppresses loading-induced deformations within the bone and thus reduces the mechanical stimulus that would increase the bone size (Järvinen and others 2003b).

In this investigation, the influence of castration on skeletal status was examined by comparing castrated and intact male rabbits. Low androgen levels resulting from bilateral orchiectomy are known to predispose to osteoporosis and osteoporotic fractures in males (Vanderschueren and others 1992, Melton and others 2003). Significant changes in cortical and trabecular bone density have been detected within 2-4 months after the operation in adult rats. These changes were more rapid and severe in growing animals (Vanderschueren and others 1992, Prakasam and others 1999). In our study, no significant differences were noted, even though 14 out of 19 male rabbits were castrated at least 4 months before this survey. It is possible that physical activity may restore bone mass in young orchiectomised rats (Horcajada-Molteni and others 1999). The pet rabbits in the present study may have had larger living spaces and been physically more active than the rats living in the cages in the above-mentioned study. Furthermore, the rabbits in our study were apparently healthy, whereas experimental animals may suffer from severe health disorders, such as renal insufficiency as was observed in the rat study of Prakasam and others (1999). Renal insufficiency is known to have adverse effects on bone health.

The influence of oestrogen deficiency on bone after ovariectomy or ovariohysterectomy could not be investigated due to the small number of neutered females in this study (n = 5). Further research is needed to evaluate the influence of neutering on the skeletal status in pet rabbits.

A preliminary investigation of impact of dental disease on bone parameters was performed because the prevalence of dental disease was high (about 44%) in the present rabbit population. The aetiology and pathophysiology of dental disease in rabbits is unclear and probably multifactorial (Jekl and Redrobe 2013). Metabolic bone disease may be one cause of PSADD and subsequent changes in bone (Harcourt-Brown 2007). Given the systemic nature of metabolic bone diseases, it was anticipated that decreased bone mineral density might be seen in the tibia. However, no association between decreased bone mineral density in the tibia and dental disease was found. Most of the rabbits showed the initial stages of PSADD (grade 2), whereas only 10 displayed more advanced stages (grades 3-5). Accordingly, the results may have been confounded by the small sample size of rabbits with more advanced grades of PSADD. It is also possible that bone density in the tibia did not reflect bone density in the skull or jawbones. In general, while rabbits have faster skeletal change and bone turnover (bone formation rate, BFR) than many other species, there are differences in BFR between bones (Richards et al. 2007, Ernst et al. 2012). About 6% higher bone volume to total volume (~bone density) was published in the cortex of rabbit tibia than in the alveolar cortical bone of the mandible, while BFR was almost twice as high in the mandibular site compared to the tibia (Ernst et al 2012). Because the present study was a cross-sectional study, it was not possible to know the exact appearance of dental changes or monitor their progression. As dental changes are irreversible, it is possible that the dental disease appeared at an earlier age but changes in rabbits' diet, husbandry and health masked and association between dental disease and bone parameters. Thus a prospective study of rabbits with verified progression of the dental disease is warranted to account for more detailed information on this topic.

This study has some limitations. Participation in this study was voluntary and some owners were not willing to let their healthy pet rabbits to be sedated for research purposes and their rabbits were not included in the study. This rendered the study population subject to selection bias and limited the sample size and statistical power in the subgroup analyses. As has been previously shown by Mäkitaipale and others (2015), most of the pet rabbits considered healthy by their owners had actual health concerns on physical or radiographic examination. The proportion of health concerns accumulated with age, a fact that limited the number of apparently healthy rabbits in this study. Proper positioning of the scanned bone in terms of the X-ray beam is essential for the accuracy of pQCT measurements, which was ensured here by careful palpation. The validity of scanning was controlled by the image quality, and images with clear movement artefacts were excluded. The 140 mm gantry opening limits the size of the measured object, e.g., the rabbit body. Small flat skull bones and the prominent, individually variable shaped teeth make the use of pQCT challenging in mildly sedated rabbits. Therefore the tibia was chosen the only target bone in the present study, and vertebral and skull bones, as pertinent they may be, were not investigated.

In conclusion, this study of tibial bone parameters in Finnish pet rabbits, showed that there was no trend for age-related decrease in trabecular or cortical bone density within the studied age range up to 6 years. Neither sex, castration nor dental disease in pet rabbits was associated with decreased tibial bone density. Further research is needed to evaluate the influence of geriatric age, ovariohysterectomy, more advances stages of PSADD, nutrition and physical activity on skeletal status and fracture risk in pet rabbits.

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DISCLOSURE

The authors declare no conflict of interest related to this report.

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Table 1. Pearson correlation coefficients between bone parameters and clinical characteristics in pet rabbits. Statistical significance (p-value) is given in parentheses.

Characteristics	Age	Body weight	Tibial length
Distal tibia			
N	84	81	87
Total area (mm^2)	-0.163 (0.14)	**0.807 (<0.001)**	**0.861 (<0.001)**
Trabecular density (mg/cm^3)	0.035 (0.76)	**-0.478 (<0.001)**	**-0.510 (<0.001)**
Cortical density (mg/cm^3)	-0.073 (0.52)	**0.269 (0.016)**	0.152 (0.166)
Cortical area (mm^2)	-0.193 (0.20)	**0.875 (<0.001)**	**0.841 (<0.001)**
Cortical to total area ratio	-0.109 (0.33)	-0.184 (0.102)	**-0.285 (0.008)**
Strength-strain index (mm^3)	**-0.224 (0.043)**	**0.907 (<0.001)**	**0.863 (<0.001)**
Tibial shaft			
N	84	81	87
Total area (mm^2)	**-0.280 (0.016)**	**0.912 (<0.001)**	**0.861 (<0.001)**
Cortical density (mg/cm^3)	**0.344 (0.003)**	0.132 (0.271)	0.004 (0.972)
Cortical area (mm^2)	-0.158 (0.310)	**0.903 (<0.001)**	**0.820 (<0.001)**
Cortical to total area ratio	-0.071 (0.553)	**-0.257 (0.030)**	**-0.354 (0.002)**
Strength-strain index (mm^3)	**-0.271 (0.021)**	**0.932 (<0.001)**	**0.857 (<0.001)**

Table 2. Descriptive unadjusted data for intact female and male pet rabbits and group comparison based on weight- and age-adjusted general linear model (GLM) analysis of covariance (ANCOVA).

Characteristics	**Female Mean (SD)**	**Male Mean (SD)**	**ANCOVA* p value**
Age (yrs) ‡	2.6 (2.3) *37*	2.3 (1.6) *23*	0.592
Weight (kg) ‡	2.3 (1.2) *33*	2.4 (1.1) *25*	0.812
Tibial length (mm) ‡	89.2 (14.6) *38*	91.1 (13.7) *25*	0.611
Distal tibia			
N	32	23	
Total area (mm^2)	40.3 (14.4)	41.0 (16.8)	0.934
Trabecular density (mg/cm^3)	80.5 (28.0)	71.4 (28.8)	0.167
Cortical density (mg/cm^3)	1181.2 (93.1)	1194.7 (118.0)	0.618
Cortical area (mm^2)	18.0 (5.3)	18.1 (4.7)	0.778

Cortical to total area ratio	0.5 (0.1)	0.5 (0.1)	0.912
Strength-strain index (mm^3)	44.1 (23.3)	44.6 (23.6)	0.851
<u>Tibial shaft</u>			
N	32	23	
Total area (mm^2)	29.3 (11.2)	30.9 (10.2)	0.555
Cortical density (mg/cm^3)	1407.5 (46.7)	1405.3 (56.1)	0.862
Cortical area (mm^2)	19.1 (6.7)	19.6 (5.7)	0.926
Cortical to total area ratio	0.7 (0.1)	0.6 (0.1)	0.221
Strength-strain index (mm^3)	41.7 (24.2)	43.7 (20.9)	0.955

‡ *Number of rabbits in italics*
* *Weight and age used as covariates*

Table 3. Descriptive unadjusted data for castrated and intact male rabbits and group comparison based on weight- and age-adjusted general linear models (GLMs) analysis of covariance (ANCOVA).

Characteristics	Castrated male rabbits Mean (SD)	Intact male rabbits Mean (SD)	ANCOVA* p-value
Age (yrs) ‡	2.7 (1.3) *19*	2.3 (1.6) *23*	0.370
Weight (kg) ‡	2.2 (1.0) *18*	2.3 (1.1) *25*	0.618
Tibial length (mm) ‡	88.7 (12.5) *19*	91.1 (12.5) *25*	0.566
Distal tibia			
N	18	23	
Total area (mm^2)	36.6 (15.1)	41.0 (16.8)	0.433
Trabecular density (mg/cm^3)	74.4 (18.7)	71.4 (28.8)	0.828
Cortical density (mg/cm^3)	1245.7 (83.2)	1194.7 (118.0)	0.158
Cortical area (mm^2)	18.0 (5.0)	18.1 (4.7)	0.506

Cortical to total area ratio	0.5 (0.9)	0.5 (0.1)	0.263
Strength-strain index (mm^3)	42.2 (24.4)	44.6 (23.6)	0.853

Tibial shaft

N	13	21	
Total area (mm^2)	25.3 (4.1)	30.9 (10.2)	0.560
Cortical density (mg/cm^3)	1426.4 (32.8)	1405.3 (56.1)	0.320
Cortical area (mm^2)	16.7 (2.6)	19.6 (5.7)	0.698
Cortical to total area ratio	0.7 (0.0)	0.6 (0.1)	0.647
Strength-strain index (mm^3)	33.1 (7.6)	43.7 (20.9)	0.773

* *Weight and age used as covariates*

† *Includes both females and males*

‡ *Number of rabbits in italics*

FIGURE LEGENDS

Figure 1.

Positioning of a pet rabbit for right tibia scanning using peripheral quantitative computed tomography (pQCT, Stratec XCT 2000 Research+, Stratec Medizintechnik GmbH, Pforzheim, Germany).

Figure 2.

A rabbit right hind limb positioned on the gantry opening of the peripheral quantitative computed tomography (pQCT, Stratec XCT 2000 Research+, Stratec Medizintechnik GmbH, Pforzheim, Germany) using the forearm holder provided by the manufacturer.

Figure 3

A. A sagittal 64-slice computed tomography (Light-Speed VCT, GE Healthcare, Little Chalfont, United Kingdom) image of a rabbit tibia, showing distal and shaft measurement sites for pQCT measurements (green lines).

B. A peripheral quantitative computed tomography (pQCT) (Stratec XCT 2000, Stratec Medizintechnik GmbH, Pforzheim, Germany) cross-sectional image at the distal (4 %) measurement site.

C. A pQCT cross-sectional image at the shaft (50 %) measurement site (tibia is separated from fibula, using the white line).

Figure 4.

Scatter plot between age and tibial shaft cortical bone density (mg/cm^3) in healthy pet rabbits. White boxes represent intact males, yellow boxes castrated males, red dots intact females, yellow dots neutered females and shaded area mean ± 2SD.

Figure 5.

Scatter plot between age and tibial shaft cortical bone density (mg/cm^3) in healthy pet rabbits and rabbits with dental disease. Blue dots represent healthy pet rabbits, red dots rabbits with dental disease and shaded area mean ± 2SD.